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The Effect of Aspirin on Endothelial Progenitor Cell Biology: Preliminary Investigation of Novel Properties

Junyang Lou, MD, PhD^{†, **}, Thomas J. Povsic, MD, PhD^{†, **}, Jason D Allen, PhD[†], Stacie D. Adams, BS, Shelley Myles, BS, RN[†], Aijing Z Starr, MS[†], Thomas L. Ortel, MD, PhD[‡], and Richard C. Becker, MD^{†, §}

[†]Department of Medicine, Division of Cardiology, Duke Clinical Research Institute, Duke University School of Medicine, Duke University Medical Center

[§]Department of Medicine, Division of Hematology, Duke University School of Medicine, Duke University Medical Center

Abstract

Atherosclerosis develops in an environment of endothelial injury and inflammation. Circulating endothelial progenitor cells (EPCs) are required for vascular repair and restoration of normal endothelial function. We tested the hypothesis that the nonselective cyclooxygenase (COX) inhibitor aspirin exerts an effect on circulating EPCs.

Methods—As part of a larger study evaluating the effect of aspirin dose in primary and secondary prevention, subjects (n=32) were assigned randomly to either 81mg or 325mg aspirin daily for two months, and circulating mononuclear cells were enumerated at the beginning of the study and after 2 months using fluorescent antibodies against CD34 and CD133 as well as based on aldehyde dehydrogenase (ALDH) activity. Brachial artery endothelial function via flow-mediated dilation (BAFMD) and light transmittance platelet aggregometry in response to physiologic agonists was also determined.

Results—Subjects taking aspirin at the time of study entry had a lower numbers of CD133+/34+ cells compared to those not previously exposed (0.01% vs. 0.05% of MNCs, $P<0.03$). After 2 months, subjects randomized to 81 vs. 325 mg of ASA had no significant differences in the median numbers of EPCs, although mean numbers trended lower in the high dose group. Patients on chronic ASA therapy continued to have lower numbers of EPCs. Similar effects were observed in CD34 and CD 133 single-positive cells, as well as ALDH^{br} cells. BAFMD did not differ nor change significantly over time between aspirin dose groups. All patients had decreased ex vivo platelet aggregation in response to arachidonic acid and ADP stimulation.

Conclusions—Our preliminary studies suggest that aspirin exerts a time-dependent effect on circulating EPCs. Short-term exposure to differing doses of ASA had indeterminate effects on EPCs levels, suggesting that time of ASA exposure may play a more important role than dose. Determining the responsible mechanism(s) and the overall clinical relevance of these findings will require further investigation.

Correspondence: Richard C. Becker, MD, Professor of Medicine, 2400 Pratt Street, Durham, NC 27705, becke021@duke.edu.

^{**} Contributed equally to the study and manuscript

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Introduction

Advanced atherosclerotic coronary artery disease (CAD) represents a leading cause of death, disability, and health care expenditures worldwide. Despite well documented progress in prevention, CAD is expected to exert a profound global impact over the next 30 years- based in large part to emerging epidemics of obesity, inactivity, diabetes, smoking and concomitant environmental air pollution.

While numerous clinical trials support the clinical benefit of aspirin as a means to reduce cardiovascular events in moderate-to-high risk populations, the most safe and effective daily dose remains poorly defined [1]. Moreover, the mechanism(s) of benefit, above and beyond the well-characterized platelet-inhibiting effects, are not fully understood.

The Aspirin Study was a proof-of-concept, pilot investigation designed to determine the feasibility of an Internet-based approach to conducting clinical research that incorporated patient-initiated follow-up and data entry. A prospectively-designed mechanistic substudy was undertaken to investigate the effect of aspirin on endothelial progenitor cell biology and brachial reactivity-determinates of vascular repair and responsiveness. The Aspirin mechanistic substudy will be the focus of the current report.

Study Methods

Aspirin Study: Population and Enrollment—The Aspirin pilot study was designed to compare two doses of aspirin (81 mg and 325 mg daily) using an interactive, Internet-based system of communication between enrolled patients and the Aspirin study investigators. Subjects 18 years of age at risk for CHD or those with established CHD, no intolerance or allergy to an aspirin dose 325 mg/day, and access to the Internet were eligible for study participation. Subjects were excluded for the following reasons: ingestion of a non-steroidal anti-inflammatory drug (other than aspirin) within the prior 10 days, thrombocytopenia ($< 100,000$ per mm^3) or an inability to comply with the study protocol.

Blood Sampling—Blood samples were collected by an experienced phlebotomist using either a 21 or 23- gauge butterfly needle. All subjects were at rest for at least 30 minutes before sample procurement. Prior to collecting study samples, one 3mL red top tube was drawn and discarded. Each study tube was completely filled, gently inverted 6–8 times, labeled, double-bagged, placed in a metal container at ambient temperature and transported by a study coordinator within 60 minutes of procurement to the laboratory for analysis.

Determination of EPC numbers—EPC numbers were simultaneously assayed using analysis of cell surface expression of CD133, C34, and vascular endothelial growth factor receptor-2 (VEGFR-2) as well as on the basis of ALDH (aldehyde dehydrogenase) activity. Peripheral blood mononuclear cells were isolated from 7–10 ml of blood by layer onto cell preparation tubes (CPT tubes, Becton Dickinson). Tubes were spun at 1800 rcf for 30 minutes and the buffy coat transferred to a 50 ml conical tube. Recovered cells were washed with PBS-1% BSA (30 mls), recovered after centrifugation at 500 rcf for 10 min., re-suspended in PBS-1% BSA, counted and dispersed for determination of EPC numbers.

Identification of EPCs by ALDH expression—Total human peripheral blood was analyzed for the relative content of cells with low orthogonal light scatter and high ALDH activity content (SSC^{lo} ALDH^{br} cells) [2]. Briefly, 4×10^6 cells were aliquoted for analysis into an Aldecou tube containing 2 ml of Aldecou buffer (Aldagen Inc, Durham NC). Immediately after addition of the cells, 500 μL was transferred to a tube containing diethylaminobenzaldehyde (DEAB), a potent inhibitor of aldehyde dehydrogenase activity

(10 uM). After 30 minutes at 37° C, the cells were centrifuged, placed on ice and flow cytometry was performed.

Identification of EPCs by expression of cell surface markers—Isolated mononuclear cells were washed with Iscove's Modified Dulbecco's Medium containing 2% fetal calf serum (IMDM+2%), concentrated to 10^7 cells/ml in 80 uL IMDM+2%. Non-specific antibody binding was blocked with FcR blocking reagent (Miltenyi Biotec, 10 uL) for 10 min. Using CD133-APC (Miltenyi Biotec), CD34-FITC (Miltenyi Biotec), and VEGFR2-PE (R and D Biosystems) allowed simultaneous staining for all three key cell surface markers. Dead and dying cells will be excluded using staining with 7-AAD (1 ug/ 10^6 cells, Invitrogen) added just prior to performance of flow cytometry.

Flow Cytometry—Flow cytometry was performed by trained technicians blinded to patient identity using an LSR II flow cytometer (BD Biosciences, San Jose, CA) and analyzed using Flow Jo software (Treestar, Costa Mesa, California). Quality control measures were performed daily using BD Comp Beads (Becton Dickinson) incubated with each antibody.

Analysis was performed in a blinded fashion. Dead and dying cells were excluded based on staining with 7-AAD. The numbers of CD34⁺, CD133⁺, and VEGFR-2⁺ cells were identified as sub-populations of a lymphocyte/monocyte gate, determined on the basis of light front and side scatter characteristics. The number of cells staining for combinations of these markers was determined by gating on individual markers in sequential fashion. Reported frequencies were expressed as percentages of the mononuclear cell population.

Measurement of Brachial Artery Flow-Mediated Dilation (BAFMD)—Prior to imaging, subjects were instructed to hold medications, fast and refrain from exercise for 12hr and alcohol for 48hr. All vascular imaging was performed between 8am and 11am, on the left arm, with the subject in a supine position with the forearm extended and slightly supinated. Brachial artery assessments were obtained using high-resolution ultrasound and a 7.5MHz linear array transducer (Accuson, Sequoia 512), at baseline (following 10min of supine rest), during five minutes of forearm occlusion, and continuously on r-wave trigger for 2 minutes following cuff release (hyperemia) s previously described [3, 4]. **Arterial** diameter and blood flow was measured from the digital recordings. Diameters were determined from the anterior to posterior interface between the media and adventitia (the "m" line), rather than between intimal layers. The percent change in brachial artery diameter was calculated by ... *((peak post-hyperemia diastolic diameter – baseline diastolic diameter)/ baseline diastolic diameter)*100*

Reproducibility of the BAFMD technique has previously yielded average mean differences in brachial artery diameter change for days, testers, and readers of 1.91%, 1.40%, and 0.21mm respectively, with intra-class correlation coefficients of 0.92, 0.94, and 0.90, respectively [4].

Analytical Methods

BAFMD, endothelial progenitor cell (EPC) number and platelet aggregometry experiments were conducted at baseline and after 2 months of aspirin treatment. Due to the small sample size, non-parametric tests were employed for analysis. The effect of aspirin treatment after 2 months was determined by Wilcoxon Signed Rank test in all subjects. The effect of aspirin dose was assessed by Wilcoxon Rank Sum test in all subjects. Treatment effect was also examined after adjusting two baseline covariates- age and body mass index (BMI) using analysis of covariance (ANCOVA) method with rank transformation [5]. The correlation

between BMI and BAFMD, EPC number and platelet aggregation was assessed by examining Spearman coefficients.

Data is also presented as mean $\pm$ standard error of the mean. Comparison of means is performed using a Student's t-test.

Results

Substudy population

A total of 33 individuals participated in the Aspirin Pilot Substudy of which EPC data for 26 patients is available at both study entry and after the 2 month follow-up visit. The 26 patients include 9 patients not taking aspirin at study entry, while 17 were on various doses of aspirin. On study entry, patients were randomized to 325 mg (n=7) or 81 mg (n=19) of aspirin.

The median (IQR) age was 54 (45,63) years (range 28 to 81 years). Thirteen subjects (39%) were male. Other patient characteristics are listed in Table 1. A majority (85%) were non-smokers, did not have diabetes (96%), and lacked hypertension (69%) or hypercholesterolemia (58%).

Patients previously treated with aspirin were older (median age 63 [IQR: 54,72] vs. 45 [IQR: 44,49], more likely to be male (47.1% vs. 22.2%), have hypertension (41.2% vs. 11.1%), hypercholesterolemia (58.8 vs. 11.1%), and to have had prior CABG (29.4% vs. 0%).

Endothelial Progenitor Cells

At study entry, aspirin-naïve subjects had significantly more CD133⁺/34⁺ double-positive cells than individuals already on aspirin (median 0.014 [IQR: 0.010–0.064%] vs. median of 0.007 [IQR: 0.005–0.013%], $p = 0.087$, mean numbers $0.047 \pm 0.018\%$ vs. $0.012 \pm 0.004\%$, $p < 0.05$, Figure 1). A similar decrease was observed using an ALDH assay (median 0.065 [IQR 0.052–0.142%] vs. median of 0.050 [IQR: 0.035–0.072%], $p = 0.11$, mean numbers $0.134 \pm 0.056\%$ vs. $0.054 \pm 0.010\%$, $p = 0.07$). Comparisons of the two groups based on VEGFR⁺, CD34⁺ and CD133⁺ single-positive cells yielded similar trends (Table 2).

After two months of daily administration, subjects receiving aspirin 81mg/d had a lower number of CD133⁺/34⁺ double-positive cells than subjects given 325mg/d (mean 0.022 ± 0.004 vs. 0.012 ± 0.003 , $p = 0.21$, median 0.010 [IQR: 0.006–0.015%] vs. 0.013 [IQR: 0.012–0.079%]) [Table 3]. The same trend was observed when EPCs were identified on the basis of ALDH activity (mean 0.089 ± 0.028 vs. 0.040 ± 0.011). Aspirin-naïve subjects on study entry continued to demonstrate higher levels of EPCs after study completion, regardless of the dose of ASA taken during the course of the study (median 0.021% [IQR: 0.009–0.054%] vs. 0.010 [IQR: 0.007–0.020], $p = 0.13$) [Table 4]. Again similar trends were observed for ALDH^{br} (median 0.070% [IQR: 0.055–0.103%] vs. 0.049 [IQR: 0.032–0.075], $p = 0.12$), CD133⁺ cells (median 0.075% [IQR: 0.068–0.080%] vs. 0.052 [IQR: 0.036–0.071], $p = 0.13$), and CD34⁺ cells (median 0.118% [IQR: 0.086–0.137%] vs. 0.069 [IQR: 0.051–0.091], $p = 0.05$) [Table 4].

Female sex correlated inversely with EPC number as determined by CD133/34 ($r = 0.47$; $p = 0.01$). There was a statistically insignificant inverse correlation between aspirin dose and the numbers of EPCs defined on the basis of ALDH activity ($r = -0.033$; $p = 0.09$).

Brachial Artery Endothelial Function via Flow Mediated Dilatation (BAFMD)

Following 2 months of treatment, subjects receiving aspirin 81mg/d had a maximal percent change of 5.12 [IQR: 4.09, 5.66]. In contrast, subjects receiving aspirin 325mg/d

experienced a maximal percent change of 4.99 [IQR: 2.51, 7.93]. Aspirin-naïve patients had a maximum change of 5.05 [IQR: 3.33, 5.59]. In summary, there were no significant differences in BAFMD between patients randomized to 81 mg vs. 325 mg of aspirin, or after treatment of aspirin naïve patients for 2 months with aspirin therapy.

Platelet Aggregation

The median aggregation to 2uM ADP, 10uM ADP and 0.5 mM arachidonic acid among patients native to aspirin at enrollment are 46 [IQR 36,64.5] %, 68 [IQR 61,75]% and 2 [IQR 1.5, 4]% respectively. After two months of treatment, aggregation to was 38 [IQR: 33.25, 65.5] %, 68.5 [IQR: 62,75.25]%, and 2.5 [IQR: 0.75,3.25]% respectively. After 2 months of treatment, platelet aggregation to 2uM ADP, 10uM ADP and 0.5 mM arachidonic acid for all 26 patients was 37.5 [IQR: 31, 46]%, 67 [IQR: 57, 69]% and 2.0 [IQR: 1.0, 3.0] %, respectively. There were no significant differences in the degree of platelet inhibition between subjects receiving 81mg and 325 mg aspirin daily.

Discussion

Aspirin is the most widely used medicinal agent in the world, with 20 to 30 billion tablets consumed annually in the United States alone, with proven efficacy in the prevention of cardiovascular events; however, the best dose to maximize efficacy is of some debate. We designed the aspirin pilot study to assess the feasibility of using an internet-based approach to inexpensively and efficiently conduct a clinical trial of aspirin dosing in preparation for a planned large clinical trial to assess the effect of aspirin dosing on clinical endpoints.

The description of cells in the circulation capable of endothelial differentiation and vascular repair altered our conception of vascular disease from a progressive disorder of accumulating vessel injury to one in which ongoing damage is counter-balanced by inherent reparative processes. As part of this study, we conducted a sub-study to investigate the effects of aspirin dosing on a variety of biological parameters, including endothelial progenitor cells, to determine the effect of aspirin administration and dosing on the numbers of EPCs in the circulation. Although our sample size was limited, we showed intriguing trends toward lower numbers of EPCs in patients on long-term aspirin therapy. In addition, we observe trends towards lower numbers of EPCs in patients on higher dose (325 mg) of daily aspirin.

Endothelial progenitor cells have the innate ability to proliferate, migrate and differentiate into cells that line the vascular lumen, where they participate in vascular repair and the maintenance of a surface that resists thrombosis and inflammation. Although vessel wall injury is a recognized stimulus for EPC release, patients at risk for vascular events, as determined by the Framingham risk score, have a reduced number of circulating progenitor cells [6, 7]. This paradox, which is also accompanied by attenuated BAFMD [6], supports an acquired state of impaired vessel wall reparative capacity and functional reserve.

The effect of aspirin on circulating EPC number and function is an area that has received limited attention. Santilli and colleagues measured endothelial colony-forming cells (ECFC) in 37 patients with polycythemia vera, a myeloproliferative disorder associated with altered endothelial cell repair and a predisposition for thrombosis, who had received low-dose aspirin for 8 weeks. Compared with 12 healthy controls, patients were found to have a 7-fold lower number of EPCs [8]. In a modified Boyden chamber assay, Hu reported that aspirin promoted EPC migratory and adhesive capacity at concentrations between 0.1 and 100uM/L, and prevented cell senescence at concentrations between 50 and 100uM/L [9]. This range of concentrations did not, however, affect EPC proliferation, apoptosis or eNOS expression. The maximal aspirin plasma concentration following a dose of 75mg is approximately

40uM/L[10]. A larger dose increases plasma concentrations approximately 3-fold. Prior investigations of aspirin on EPC biology have been conducted either *in vitro* or *ex vivo*. To the best of our knowledge, the effect of daily aspirin administration on the number of circulating EPCs *in vivo* has not been reported previously; however, in an observation database of 282 patients in whom EPCs were similarly analyzed, we also observed significantly lower levels of ALDH^{br} cells, with trends toward lower numbers of CD34 and CD34/CD133⁺ cells, in patients who were on chronic aspirin therapy (Povsic, personal communication). This data should be interpreted with caution, given its observational nature and the association between aspirin use and multiple other factors associated with more advanced vascular disease.

A property of progenitor cells derived from multiple lineages is their high level of aldehyde dehydrogenase (ALDH) activity [11–15]. Accordingly, the use of ALDH activity may form the basis for a novel EPC assay which is more reliable and reproducible than previously described methods [2, 16]. The numbers of ALDH^{br} cells correlate more strongly with age and extent of CAD than EPCs identified by previously described assays, suggesting that ALDH activity may identify cells more directly involved in vascular repair than other EPC populations. We and others have shown that ALDH^{br} cells obtained from human bone marrow display a greater capacity for endothelial differentiation and outgrowth than bone marrow CD133⁺CD34⁺ cells [2, 13]. We observe similar trends between ALDH^{br} cells and EPCs identified on the basis of cell surface CD133 and CD34 expression, consistent with prior studies [2, 16].

Aspirin Dose and EPC Biology

Our preliminary studies suggest that aspirin may decrease the number of circulating EPCs; furthermore, when aspirin-naïve patients began taking aspirin, their EPC numbers decreased as well. We postulate that the responsible mechanism may involve aspirin-mediated inhibition of COX-dependent pathways and offer the following biology-based explanation. There are data showing that aspirin-mediated COX-2 inhibition suppresses angiogenesis and may trigger apoptosis. High levels of COX-1 protein expression and PGI₂ synthesis have been identified in EPCs [17], and subsequent inhibition with indomethacin and siRNA, respectively, reduces proliferative capacity. Herrler and colleagues have shown that EPC-mediated PGE-2 synthesis is vital for their production, trafficking and functionality[18]. Colleselli et al demonstrated that rat circulating EPCs expressed COX-1 and COX-2. Further, cultured EPCs exposed to various COX inhibitors, including aspirin, respond with a significant suppression of proliferation that is dose-dependent [19].

A question raised by our observations is whether relatively high doses of aspirin would potentially exert a detrimental effect, or whether the reduced number of EPCs reflects a decreased vascular stimulus for EPC release. While more in-depth investigation is needed, individuals receiving aspirin 325mg daily, particularly with increasing age, were found to have fewer EPCs by several distinct methods of determination. Our observations indicate an approximate doubling of EPC numbers in aspirin naïve patients as compared with those taking aspirin. To put this in context, short term statin administration has been reported to result in an approximate 4-fold increase in circulating EPC numbers [7, 20], although these findings have not been uniformly replicated [21].

Other investigators [9, 22] have reported that high concentrations of aspirin are associated with an inhibitory effect on EPC migration, adhesion and proliferation. The basis for the dichotomous relationships for low and high aspirin plasma concentrations on EPC biology is unknown at the present time, but warrants further study.

The exact mechanisms responsible for this effect of aspirin remain to be determined. Aspirin irreversibly inhibits COX-1 (found within platelets and vascular endothelial cells) and modifies the enzymatic activity of COX-2 (found within inflammatory cells and vascular endothelial cells). Because aspirin more selectively inhibits COX-1 than COX-2 activity, its ability to prevent platelet aggregation is seen at relatively low doses, compared with the drug's potential anti-inflammatory and fibrin strand assembly modulating effects, which may require much higher doses (and salicylate concentrations) [23].

Previous studies have suggested that COX-2 inhibition may in particular affect EPC mobilization and function [19]. This raises the intriguing possibility that the selectivity of drug induced COX-1 and -2 inhibition on EPC biology may impact clinical events observed with more selective COX-2 inhibitors.

Our study has limitations, including the confounding that is inherent to the use of aspirin amongst subjects prior to enrollment in the current study, the small sample size of the current study population, and the lack of normally distributed EPC numbers, which makes statistical analysis less sensitive. While the lower EPC numbers observed in aspirin users on study entry might be attributable to the higher levels of cardiac risk factors in these patients, it should be noted that the presence of these risk factors might also be expected to impact statin use, which increases circulating EPCs [7, 24], mitigating or even reversing any expected effect of such risk factors on EPC numbers. Additionally, we observed a drop in the levels of EPCs in the ASA naïve patients after 2 months of ASA therapy, while ASA users showed no difference in follow-up, as might be expected. This suggests that ASA may have a direct effect on the biology of circulating EPCs.

These findings should be used to corroborate other reports [9, 19], and suggest this as topic worthy of future investigation. Such studies should target not only the relationship between aspirin, dose, and EPC numbers, but might explore the relationship between other COX-2 inhibitors, the selectivity for COX-2 inhibition, and effects on EPC numbers.

Conclusions

We have shown in preliminary studies that aspirin use is associated with decreases in the number of circulating EPCs in human subjects. The effect may be either dose and/or time - dependent. BAFMD was not affected by aspirin administration at the doses tested. The failure of aspirin doses above 81mg daily to exert added benefit among patients at risk for cardiovascular events [1, 25, 26] while by no means proof of harm, further heightens interest for investigating the full spectrum of biological effects attributable to aspirin.

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Figure 1. Comparison of EPC levels as determined based on cell surface expression of CD133-CD34 (left panel) and ALDH activity (right panel).

Table 1

Patient Characteristics

	All Patients	Aspirin Naïve (n=9)	Aspirin Users (n=17)
Age, median (IQR]	54 (46, 64]	45 (44, 49]	63 (54, 72]
Male, No. (%)	10 (38.5]	2 (22.2]	8 (47.1]
Current Smoking, No. (%)	4 (15.4]	1 (11.1]	3 (17.6]
Diabetes, No. (%)	1 (3.8]	0 (0.0]	1 (5.9]
Hypertension, No. (%)	8 (30.8]	1 (11.1]	7 (41.2]
Hypercholesterolemia, No. (%)	11 (42.3]	1 (11.1]	10(58.8]
Prior CABG, No. (%)	5 (19.2]	0 (0.0]	5 (29.4]
Family History of CAD, No. (%)	10 (38.5]	3 (33.3]	7 (41.2]

TABLE 2A and B

Levels of EPCs at Study Entry Amongst Aspirin naïve and Aspirin Using Subjects

A. Mean EPC numbers					
Cell Type	Aspirin Naïve		Aspirin Users		
	Mean	SEM	Mean	SEM	p-value
CD133 ⁺ -CD34 ⁺	0.047	0.018	0.012	0.004	0.02
ALDH ^{br}	0.134	0.056	0.054	0.01	0.07
CD133 ⁺	0.2353	0.1109	0.1602	0.0725	0.56
CD34 ⁺	0.176	0.066	0.1735	0.088	0.98

B. Median EPC numbers					
Cell Type	Aspirin Naïve (n=9]		Aspirin Users (n=17]		
	median	IQR	median	IQR	p-value
CD133 ⁺ -CD34 ⁺	0.014	0.010–0.064	0.007	0.005–0.013	0.087
ALDH ^{br}	0.065	0.052–0.142	0.050	0.035–0.072	0.107
CD133 ⁺	0.120	0.049–0.184	0.082	0.054–0.130	0.632
CD34 ⁺	0.130	0.062–0.180	0.084	0.068–0.103	0.490

TABLE 3 A and B

Levels of EPCs at Study End by ASA Dose

A. Mean EPC numbers					
Cell Type	81 mg (n=19)		325 mg (n=7)		p-value
	Mean	SEM	Mean	SEM	
CD133 ⁺ -CD34 ⁺	0.025	0.009	0.012	0.003	0.40
ALDH ^{br}	0.089	0.028	0.04	0.011	0.31
CD133 ⁺	0.0679	0.011	0.0412	0.0073	0.17
CD34 ⁺	0.0857	0.0178	0.0654	0.0047	0.50

B. Median EPC numbers					
Cell Type	81 mg (n=19)		325 mg (n=7)		p-value
	median	IQR	median	IQR	
CD133 ⁺ -CD34 ⁺	0.010	0.006–0.015	0.013	0.001–0.050	0.69
ALDH ^{br}	0.052	0.040–0.079	0.050	0.012–0.079	0.57
CD133 ⁺	0.082	0.049–0.130	0.184	0.039–1.045	0.49
CD34 ⁺	0.074	0.062–0.103	0.130	0.084–0.185	0.13

TABLE 4

Levels of EPCs at Study End

Cell Type	Aspirin Naïve (n=9)		Aspirin Users (n=17)		p-value
	median	IQR	median	IQR	
CD133 ⁺ -CD34 ⁺	0.021	0.009–0.054	0.010	0.007–0.020	0.1306
ALDH ^{br}	0.070	0.055–0.103	0.049	0.032–0.075	0.1184
CD133 ⁺	0.075	0.068–0.080	0.052	0.036–0.071	0.1306
CD34 ⁺	0.118	0.086–0.137	0.069	0.051–0.091	0.0571